Comparative pharmacokinetics of levamisole-oxyclozanide combination in sheep and goats following *per os* administration

Cengiz Gokbulut, Hande Sultan Yalinkilinc, Dilek Aksit, Vincenzo Veneziano

Abstract

Since there is no registered anthelmintic drug available for use in goats, extra-label use of drugs is a common practice in most countries. The aim of the present study was to compare the pharmacokinetic disposition of levamisole (LVM)-oxyclozanide (OXZ) combination in sheep and goats following *per os* administration. Goats (n = 8) and sheep (n = 8) 12- to 16-months-old were used for this study. The animals received tablet formulation of LVM and OXZ combination orally at a dose of 7.5 mg/kg and 15 mg/kg body weight, respectively. Blood samples were collected by jugular vein at different times between 5 min and 120 h after drug administrations. The plasma concentrations of LVM and OXZ were analyzed by HPLC following liquid-liquid phase extraction procedures. The plasma concentrations and systemic availabilities of both LVM and OXZ in goats were lower and the plasma persistence of LVM was shorter compared with those observed in sheep. Terminal half-lives ($t_{1/2\lambda z}$) of both molecules are shorter in goats compared with those in sheep. Goats treated with LVM-OXZ combination at the recommended dose for sheep may result in a reduced efficacy, because of under-dosing, which may increase the risk of drug resistance in parasites. Increased or repeated dose could be a strategy to provide higher plasma concentration and thus to improve the efficacy against the target parasites in goats compared with sheep. However, some adverse reactions may occur since LVM has relatively very narrow therapeutic index due to its nicotine-like structure and effect.

Résumé

Étant donné qu'il n'y a aucun anthelminthique homologué disponible pour utilisation chez les chèvres, l'utilisation hors-homologation de médicaments est une pratique usuelle dans la plupart des pays. L'objectif de la présente étude était de comparer la disposition pharmacocinétique de la combinaison levamisole (LVM)-oxyclozanide (OXZ) chez les moutons et les chèvres suite à l'administration per os. *Des chèvres* (n = *8) et des moutons* (n = *8) âgés de 12 à 16 mois furent utilisés pour cette étude. Les animaux ont reçu une combinaison de comprimés de LVM et d'OXZ à une dose de 7,5 mg/kg et 15 mg/kg de poids corporel, respectivement. Des échantillons sanguins furent prélevés par ponction de la veine jugulaire à différents temps entre 5 min et 120 h suite à l'administration des médicaments. Les concentrations plasmatiques de LVM et d'OXZ furent analysées par HPLC suite à des procédures d'extraction de phase liquide-liquide. Les concentrations plasmatiques et les disponibilités systémiques de LVM et OXZ chez les chèvres étaient plus basses et la persistance plasmatique de LVM de plus courte durée comparativement à celles observées chez les moutons. Les demi-vies terminales ($t_{1/2\lambda z}$) des deux molécules sont plus courtes chez les chèvres comparativement à celles chez les moutons. Le traitement de chèvres avec la combinaison LVM-OXZ au dosage recommandé pour les moutons pourrait résulter en une efficacité moindre, dû à un sous-dosage, ce qui pourrait augmenter le risque de résistance au médicament chez les parasites. Des doses augmentées ou répétées pourraient s'avérer une stratégie pour obtenir des concentrations plasmatiques plus élevées et ainsi améliorer l'efficacité contre les parasites ciblés chez les chèvres comparativement aux moutons. Toutefois, quelques réactions indésirables peuvent survenir étant donné que le LVM a déjà un index thérapeutique assez étroit associé à sa structure et son effet apparentés à la nicotine.*

(Traduit par Docteur Serge Messier)

Introduction

Levamisole (LVM), the laevo-rotary isomer of tetramisole [2,3,5,6-tetrahydro 6–phenyl imidazo (2,1-b) thiazole] is a broad-spectrum anthelmintic drug active against most nematodes (1) and widely used in veterinary medicine. Levamisole induces spastic paralysis in the target nematodes as a result of permanent muscle contraction. Oxyclozanide (OXZ) [2,3,5-trichloro-N-(3,5-dichloro-2-hydroxyphenyl)-6-hydroxybenzamide] is a salicylanilide anthelmintic drug that mainly acts by uncoupling oxidative phosphorylation in flukes (2,3). It is used for the treatment and control of adult stages of liver flukes in large and small ruminant species (4,5). Combination of anthelmintics formulated has been used to provide positive results against resistant nematodes (6–8) and broad-spectrum control of different types of internal parasites, e.g., nematodes and tapeworms or nematodes and liver flukes in ruminants.

The extra-label use of drugs is a common practice in goats. The high prevalence of anthelmintic-resistant nematodes in goats is probably due to the extensive extra-label use of drug at the sheep doses

Department of Pharmacology, Faculty of Medicine, Balikesir University, Balikesir, Turkey (Gokbulut); Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, Turkey (Yalinkilinc); Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Balikesir University, Balikesir, Turkey (Aksit); Department of Pathology and Animal Health, Faculty of Veterinary Medicine, University of Naples Federico II, Naples, Italy (Veneziano).

Address all correspondence to Dr. Cengiz Gokbulut; telephone: + 90 266 612 14 54; fax: + 90 266 612 14 59; e-mail: cengizgokbulut@yahoo.com

Received May 22, 2013. Accepted August 31, 2013.

recommended, corresponding to a drug under-dosage. It has been reported that the metabolism of many therapeutic drugs, including anthelmintics, differs between sheep and goats (9–17). The drug molecules are more rapidly metabolized and eliminated from blood in goats compared with sheep. Hence, administration of anthelmintic drugs to goats at an ovine dosage has resulted in a reduced efficacy, because of under-dosing. This may explain the differences in the prevalence of anthelmintic resistance in nematode populations in goats compared with sheep in particular for multi-resistant strains (14,18). Therefore, the purposes of this study were to compare the pharmacokinetic disposition of LVM-OXZ combination in sheep and goats and to find out whether the dose recommended for sheep can be applied in goats following *per os* administration.

Materials and methods

Experimental animals

Eight goats [mean body weight (BW): 23.1 ± 4.2 kg] and 8 sheep (mean BW: 24.8 ± 2.9 kg) that were 12- to 16-months old were used in this investigation. They were housed and fed with wheat straw, fodder, and concentrate feed. Water was supplied *ad libitum*. This study was approved by Animal Ethic Committee of University of Adnan Menderes.

Treatment and sampling

The animals received tablet formulation of LVM and OXZ combination (Zelensin, 375 mg LVM HCl + 750 mg OXZ, Sanovel, Istanbul, Turkey) orally at a dose of 7.5 mg/kg BW and 15 mg/kg BW, respectively. Heparinized blood samples (5 mL) were collected by jugular venipuncture prior to drug administration then at 5, 10, 15, and 30 min, 1, 1.5, 2, 4, 8, 12, 16, 24, 32, 48 h and 3, 4, 5, 6, and 8 days.

Analytical procedures

The plasma concentrations of LVM and OXZ were analyzed by high-performance liquid chromatography (HPLC) following liquid-liquid phase extraction. Stock solutions (100 μg/mL) of pure standard of LVM hydrochloride and OXZ (Sigma, St. Louis, Missouri, USA) were prepared using acetonitrile and water (50:50) as the solvent. These were diluted to give 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 20 μg/mL standard solutions for plasma for calibration as standard curves and to add to drug-free plasma samples to determine the recovery of both molecules.

Extraction from plasma

Plasma concentrations of LVM and OXZ were determined by HPLC with ultraviolet detection according to methods previously described by Garcia et al (19) and Jo et al (20) with some modifications, respectively.

For LVM analysis, drug-free plasma samples (1 mL) were spiked with standard of LVM to reach the following final concentrations: 0.025, 0.05, 0.1, 0.5, 1 and 5 μg/mL. Water (1 mL) and 0.5 mL of 10 N sodium hydroxide were added to 10 mL-ground glass tubes containing 1 mL spiked or experimental plasma samples. After mixing by vortex for 15 seconds, 6 mL ethyl ether:nhexane (80:20, vol/vol), was added. The sample tubes were stoppered and shaken for 10 min on a rotary mixer. After centrifugation at 3000 × *g* for 10 min, the upper organic phase was transferred to a thin-walled 10 mL-conical glass tube and evaporated to dryness at 40°C in a rota vapor (Maxi-Dry plus, Heto, Denmark). The dry residue was reconstituted with 250 μL mobile phase. Then, the tubes were placed in an ultrasonic bath and finally, 50 μL of this solution was injected into the chromatographic system.

For OXZ analysis, drug-free plasma samples (1 mL) were spiked with standard of OXZ to reach the following final concentrations: 0.05, 0.1, 0.5, 5, 10, and 20 μg/mL. Acetonitrile (2 mL) was added, and the sample was mixed by vortex (10 s). Anhydrous sodium sulphate (1 g) was added, and the sample tubes were stoppered and shaken for 10 min on a rotary mixer. The samples were centrifuged at 3000 × *g* for 10 min thereafter, and extracted once again with 2 mL of acetonitrile. The organic layers were recovered after 20 min of centrifugation at 3000 × *g*. Then n-hexane (5 mL) was added to the combined supernatant. After shaking, the upper phase was discarded after 10 min of centrifugation at 3000 × *g*. The lower phase was transferred to a thin-walled 10 mL-conical glass tube and evaporated to dryness at 40°C in a rota vapor (Maxi-Dry plus). The dry residue was reconstituted with 250 μL mobile phase. Then, the tubes were placed in an ultrasonic bath and finally, 50 μL of this solution was injected into the chromatographic system.

High-performance liquid chromatography system

The mobile phase consisted of acetonitrile and water (2% acetic acid) (20:70, v/v) and was delivered (1100 Series, QuatPump Agilent, Waldron, Germany) at an isocratic flow rate of 1 mL/min. A nucleosil C_{18} analytical column (Luna, 4 μm, 150 mm × 4.6 mm; Phenomenex, Macclesfield, Cheshire, UK) with nucleosil C_{18} guard column was used for analysis of the molecules. Ultraviolet detection (1100 Series, Agilent, Waldron, Germany) was at a wavelength of 225 nm for LVM analysis.

Oxyclozanide were determined using same HPLC system and eluted with a mobile phase consisting of a mixture of acetonitrile and 0.1% phosphoric acid (40:60, v/v). The isocratic mode was run at a flow rate of 1 mL/min and an ultraviolet detector was operated at 300 nm.

Method validation

The analytical methods used for LVM and OXZ in plasma samples were validated before analysis of the experimental samples. The analytes were identified with the retention time of pure reference standards. Recoveries of the molecules under study were measured by comparison of the peak areas from spiked plasma samples with the areas resulting from direct injections of standard solutions. The inter- and intra-assay precisions of the extraction and chromatography procedures were evaluated by processing replicate aliquots of previously drug-free goat and sheep plasma samples containing known amounts of the drugs on different days.

The calibration graphs for LVM and OXZ were prepared (linear range 0.025 to 10 μg/mL). The slopes of the lines between peak areas and drug concentrations were determined by least squares linear regression and correlation coefficient *(r)* and coefficient of variations (CV) calculated. Linearity was established to determine the drug concentration/detector response relationship. The detection limits

of the both LVM and OXZ were established with HPLC analysis of blank plasma fortified with the standard, measuring the baseline noise at the retention time of the peak. The mean baseline noise at the peak retention time plus 3 standard deviations was defined as the detection limit (LOD). The mean baseline noise plus 6 standard deviations was defined as the limit of quantification (LOQ).

Pharmacokinetic and statistical analysis of data

The plasma concentration *versus* time curves obtained after each treatment in individual animals, were fitted with the WinNonlin software program (Version 5.2; Pharsight Corporation, Mountain View, California, USA). Pharmacokinetic parameters for each animal were analyzed using non-compartmental model analysis with extravascular input for LVM and OXZ. The maximum plasma concentration (C_{max}) and time to reach maximum concentration (t_{max}) were obtained from the plotted concentration-time curve of each drug in each animal. The trapezoidal rule was used to calculate the area under the plasma concentration time curve (AUC). The mean residence time (MRT) was calculated as:

$$MRT_{last} = AUMC_{last}/AUC_{last}$$

Terminal half-life ($t_{1/2\lambda z}$) was calculated as:

$$t_{1/2\lambda z} = -\ln(2)/\lambda_z$$

Where: λ_z represents the first-order rate constant associated with the terminal (log linear) portion of the curve.

The pharmacokinetic parameters obtained from each animal were reported as a median with interquartile ranges (Q1–Q3) and statistically compared by the Mann-Whitney U-test between sheep and goats. The values were considered significantly different at $P < 0.05$.

Results

The analytic procedures and HPLC analysis of LVM and OXZ were validated before analysis of experimental samples. Mean recoveries of LVM and OXZ from plasma were 86.31% and 74.12% with a relative SD < 10%, respectively. The limit of detections and limit of quantification for LVM and OXZ were 0.014 to 0.043 μg/mL and 0.011 to 0.032, respectively. The inter-assay and intra-assay precisions of the extraction and chromatography procedures were evaluated by processing on different days 6 replicate aliquots of drug-free sheep or goat plasma samples that contained known amounts of LVM and OXZ. The precision determined at each concentration was < 15% of the coefficient of variation, and accuracy ranged from 92% to 106% for both molecules.

Median (with Q1–Q3 ranges) pharmacokinetic parameters of LVM and OXZ combination in goats and sheep are shown in Table I with the plasma concentration *versus* time curves (Figures 1 and 2, respectively). This study indicated that the plasma dispositions of both LVM and OXZ in goats were significantly different to those observed in sheep following oral administration at same dose rates. Although no significant difference was found for maximum plasma concentration (C_{max}: 1.63 μg/mL *versus* 1.07 μg/mL), significantly larger area under the concentration *vs.* time curve (AUC: 8.75 μg.h/mL *versus* 2.09 μg.h/mL), longer terminal half-life ($t_{1/2\lambda z}$: 7.91 h *versus* 3.81 h) and mean residence time (MRT: 7.11 h *versus* 2.69 h) were observed

Table I. Median pharmacokinetic parameters with the interquartile (IQ) ranges (Q1–Q3) of levamisole (LVM) and oxyclozanide (OXZ) combination in sheep and goats following single *per os* administration (*n* = 8)

	LVM (7.5 mg/kg)		OXZ (15 mg/kg)	
Parameters	Sheep	Goat	Sheep	Goat
$t_{1/2\lambda z}$ (h)	7.91** (6.17–8.72)	3.81 (2.856–4.297)	21.74* (19.07–26.09)	18.71 (14.55–19.90)
t_{max} (h)	0.83 (0.083–0.083)	0.83 (0.083–0.250)	20.00 (12.00–24.00)	24.00 (16.00–24.00)
C_{max} (μg/mL)	1.63 (0.81–2.10)	1.07 (0.78–1.71)	11.01* (9.48–16.72)	6.68 (4.92–9.03)
t_{last} (h)	20.00* (13.00–32.00)	8.00 (8.00–12.00)	192.0* (174.0–210.0)	156.0 (120.0–186.0)
AUC_{last} (μg.h/mL)	8.75** (3.76–11.19)	2.09 (1.42–2.88)	488.70* (322.0–786.0)	309.33 (216.0–380.3)
MRT_{last} (h)	7.11** (4.71–10.51)	2.69 (2.06–3.55)	38.39 (36.33–42.92)	35.97 (34.46–40.32)

$t_{1/2\lambda z}$ — terminal half-life; t_{max} — time to reach peak plasma concentration; C_{max} — peak plasma concentration; t_{last} — time to last detectable plasma concentration; AUC_{last} — area under the (zero moment) curve from time 0 to the last detectable concentration; MRT_{last} — mean residence time.

The kinetic parameters of LVM or OXZ in sheep are significantly different ($P < 0.05$, **$P < 0.01$) from goats.

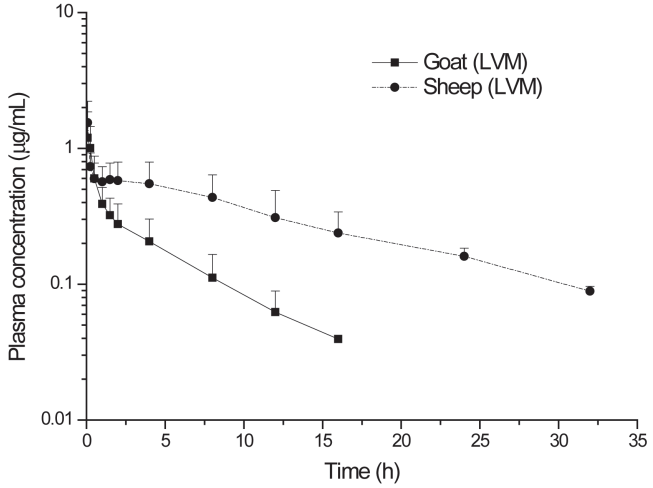

Figure 1. Plasma disposition *versus* time curves of levamisole (LVM) in goats and sheep following single *per os* administration (n = 8).

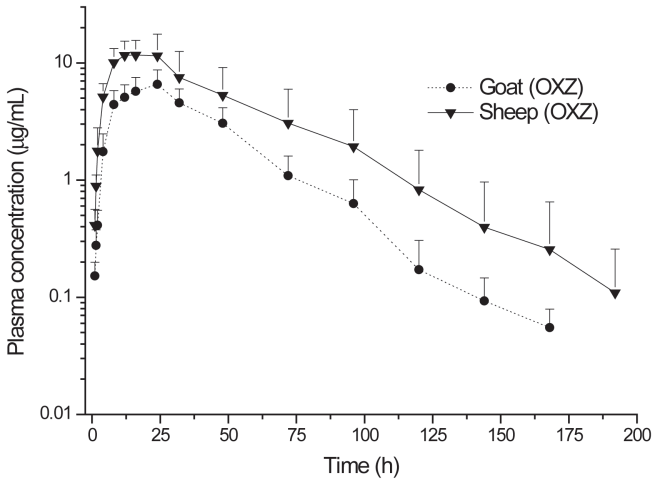

Figure 2. Plasma disposition *versus* time curves of oxyclozanide (OXZ) in goats and sheep following single *per os* administration (n = 8).

in sheep as compared with those observed in goats for LVM, respectively. In addition, C_{max} (11.01 µg/mL *versus* 6.68 µg/mL), AUC (488.70 µg.h/mL *versus* 309.33 µg.h/mL) and $t_{1/2\lambda z}$ (21.74 h *versus* 18.71 h) values of OXZ in sheep were significantly higher and longer compared with those values observed in goats, respectively.

Discussion

The plasma concentrations of both LVM and OXZ in sheep were considerably higher and the plasma persistence of LVM was longer compared with those observed in goats following oral administration. The origin of the lower plasma concentration of both molecules in goats is unclear. The most likely explanation is that goats have greater metabolic capacity and elimination capability of anthelmintic compounds than do sheep, as has been previously demonstrated with LVM (21) and other broad-spectrum anthelmintic groups such as benzimidazoles (10) and macrocyclic lactones (22–24). Moreover, it was reported that goats were better adapted to tolerate and detoxify plant toxins compared with sheep (25,26).

Levamisole rapidly reached the plasma peak concentrations at 0.08 h in sheep and goats after oral administration. These values are similar to those obtained by Galtier et al (21) in sheep (0.083 h) and goats (0.166 h). On the other hand, C_{max} (1.63 µg/mL and 1.07 µg/mL) and $t_{1/2}$ (7.94 h and 3.67 h) values of LVM observed in the present study are higher and longer than those reported by Galtier et al (21) in sheep (C_{max}: 1.06 µg/mL and $t_{1/2:}$ 1.2 h) and goats (C_{max}: 0.63 µg/mL and $t_{1/2:}$ 1.25 h), respectively. In addition, Fernandez et al (27) and Sahagún et al (28) obtained much lower plasma concentration for LVM in sheep compared with the present study following *per os* administration at same rates. The origin of the differences between studies is unclear. These differences may in part be due to differences in methodology or experimental conditions since different feeding regime, formulation, and parasitological status could have caused differences in absorption, disposition, and persistence of anthelmintic drugs in the animals. In this study, tablet formulation of LVM/OXZ combination was administered to animals, whereas Galtier et al (21), and Fernandez et al (27), and Sahagún et al (28) administered LVM as an oral drench and solution, respectively. Moreover, there was the possibility of a drug-drug interaction, due to co-administration of both drugs. However, there is no information available in the literature on this kind of interaction for LVM and OXZ.

The present study indicates that the plasma concentration of OXZ in sheep is significantly greater than that in goats. The C_{max} (13.24 µg/mL) and AUC (621.46 µg.h/mL) values of OXZ in sheep more than 2 times higher and larger than those observed in goats (6.83 µg/mL and 294.70 µg.h/mL) after *per os* administration, respectively. Moreover, terminal half-life of OXZ in goats is significantly shorter than that observed in sheep. Similar differences of OXZ as observed with LVM disposition between 2 species supports that goats have lower systemic availability and faster elimination process compared with sheep. As closantel and rafoxanide are the most widely used salicylanilide group anthelmintics, they are more extensively studied in different animal species [reviewed by Swan (29)]. Nevertheless, there is a paucity of data available in the literatures on the pharmacokinetics of OXZ in animals and this is reported first herein. A radiolabel study of ^{14}C-oxyclozanide was conducted in sheep following a single dose rate of 15 mg/kg BW formulated as a commercial drench (30). The highest plasma radioactivity of 10 to 20 µg/mL (C_{max}) was found at 8 h (t_{max}) after administration. Moreover, the plasma dispositions of rafoxanide, closantel, and OXZ in sheep following *per os* administration described by Mohammed-Ali and Bogan (31). The findings of OXZ from the present study in sheep are not similar to those obtain by Mohammed-Ali and Bogan (31). C_{max} (19.0 µg/mL), AUC (1224 µg.h/mL), and $t_{1/2}$ (153.6 h) values of OXZ were much greater and longer compared with those observed in this study, respectively. These differences are probably related to the methodology of the previous study, since the number of sampling times seems insufficient to determine the actual parameters such as C_{max} and $t_{1/2}$ values.

In conclusion, the plasma concentrations of both LVM and OXZ in goats were significantly lower and the plasma persistence of LVM was significantly shorter compared with those observed in sheep following *per os* co-administration. Goats treated with LVM-OXZ

combination at the recommended dose for sheep may result in a reduced efficacy, because of under-dosing, which may increase the risk of drug resistance in internal parasites. Increased or repeated dose could be a strategy to provide higher and more persistent plasma concentration and thus improve the efficacy against the target parasites in goats compared with sheep. However, some adverse reactions may occur since LVM has a relatively narrow therapeutic index due to its nicotine-like structure and effect.

Acknowledgments

This study was supported by The Scientific and Technical Research Council of Turkey (TUBITAK) under the COST Action project CAPARA (Goat–Parasite Interactions: from Knowledge to Control).

References

1. Thienpont D, Vanparijs OFJ, Racymaekers AHM, et al. Tetramisole (R8299), a new potent broad spectrum anthelmintic. Nature 1966;209:1084–1086.
2. Froyd G. Field trials with oxyclozanide. A new liver fluke remedy for sheep and cattle. Br Vet J 1968;124:116–125.
3. Veenendaal GH, de Waal MJ. Uncoupling activity of the anthelmintic oxyclozanide in rodents. Br J Pharmacol 1974;50:435–437.
4. Walley JK. Oxyclozanide (3,3′,5,5′,6-pentachloro-2,2′-dihydroxybenzanilide-6Zanil″) in the treatment of the liver fluke *Fasciola hepatica* in sheep and cattle. Vet Rec 1966;78:267–276.
5. Paraud C, Gaudin C, Pors I, Chartier C. Efficacy of oxyclozanide against the rumen fluke *Calicophoron daubneyi* in experimentally infected goats. Vet J 2009;180:265–267.
6. Waller PJ, Dobson RJ, Haughey KG. The effect of combinations of anthelmintics on parasite populations in sheep. Aust Vet J 1990;67:138–140.
7. Anderson N, Martin PJ, Jarrett RG. The efficacy of mixtures of albendazole sulphoxide and levamisole against sheep nematodes resistant to benzimidazole and levamisole. Aust Vet J 1991;68:127–132.
8. Miller DK, Craig TM. Use of anthelmintic combinations against multiple resistant *Haemonchus contortus* in Angora goats. Small Rumin Res 1996;19:281–283.
9. Galtier P, Escoula L, Camguilhem R, Alvinerie, M. Comparative availability of levamisole in non-lactating ewes and goats. Ann Rech Vet 1981;12:109–115.
10. Bogan JA, Benoit E, Delatour P. Pharmacokinetics of oxfendazole in goats: A comparison with sheep. J Vet Pharmacol Therap 1987;10:305–309.
11. Sangster NC, Richard JM, Hennessy DR, Collins GH. Disposition of oxfendazole in goats and efficacy compared with sheep. Res Vet Sci 1991;51:258–263.
12. Hennessy DR. Physiology, pharmacology and parasitology. Int J Parasitol 1997;27:145–152.
13. Short CR. Comparative pharmacokinetics: Sorting the sheep from the goats. Vet J 1999;158:159–161.
14. Chartier C, Pors I, Hubert J, Rocheteau D, Benoit C, Bernard N. Prevalence of anthelmintic resistant nematodes in sheep and goats in Western France. Small Rumin Res 1998;29:33–41.
15. Dupuy J, Chartier C, Sutra JF, Alvinerie, M. Eprinomectin in dairy goats: Dose influence on plasma levels and excretion in milk. Parasitol Res 2001;87:294–298.
16. Hoste H, Sotiraki S, Landau SY, Jackson F, Beveridge I. Goat-nematode interactions: Think differently. Trends Parasitol 2010; 26:376–381.
17. McKellar QA, Gokbulut C. Pharmacokinetic features of the antiparasitic macrocyclic lactones. Curr Pharm Biotechnol 2012; 13:888–911.
18. Jackson F, Coop RL. The development of anthelmintic resistance in sheep nematodes. Parasitol 2000;120:95–107.
19. Garcia JJ, Diez MJ, Sierra M, Teran MT. Determination of levamisole by HPLC in plasma samples in the presence of heparin and pentobarbital. J Liq Chromatogr 1990;13:743–749.
20. Jo K, Cho HJ, Yi H, et al. Determination of oxyclozanide in beef and milk using high-performance liquid chromatography system with UV detector. Lab Anim Res 2011;27:37–40.
21. Galtier P, Escoula L, Camguilhem R, Alvinerie M. Comparative bioavailability of levamisole in non lactating ewes and goats. Ann Rech Vet 1981;12:109–115.
22. Gokbulut C, Bilgili A, Hanedan B, Aksit, D, Aksoy AM, Turgut C. Breed-related plasma disposition of ivermectin following subcutaneous administration in Kilis and Damascus goats. Res Vet Sci 2009a;87:445–448.
23. Gokbulut C, Bilgili A, Hanedan, B, Aksit D, Aksoy AM, Turgut C. Sex-related plasma disposition of ivermectin following pour-on administration in goats. Vet Parasitol 2009b;162:342–345.
24. Gokbulut C, Cırak V, Senlik B, Aksit D, McKellar Q. The effects of different ages and dosages on the plasma disposition and hair concentration profile of ivermectin following pour-on administration in goats. J Vet Pharmacol Therap 2011;34:70–75.
25. Silanikove N, Gilboa N, Perevolotsky A, Nitsan Z. Goats fed tannin-containing leaves do not exhibit toxic syndromes. Small Rumin Res 1996;21:195–201.
26. Silanikove N. The physiological basis of adaptation in goats to harsh environment. Small Rumin Res 2000;35:181–193.
27. Fernandez M, Garcia JJ, Sierra M, Diez MJ, Teran MT. Bioavailability of levamisole after intramuscular and oral administration in sheep. New Zeal Vet J 1998;46:173–176.
28. Sahagún AM, Terán MT, García JJ, Fernández N, Sierra M, Diez MJ. Oral bioavailability of levamisole in goats. J Vet Pharmacol Therap 2001;24:439–42.
29. Swan GE. The pharmacology of halogenated salicylanilides and their anthelmintic use in animals. J S Afr Vet Assoc 1999;70:61–70.
30. EMEA Committee for Veterinary Medicinal Products, Oxyclozanide Summary Report 1 The European Agency for the Evaluation of Medicinal Products Veterinary Medicines and Inspections EMEA/MRL/340/98-FINAL. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500010748.pdf 1998. Last accessed May 16, 2014.
31. Mohammed-Ali NAK, Bogan JA. The pharmacodynamics of the flukicidal salicylanilides rafoxanide closantel and oxyclozanide. J Vet Pharmacol Therap 1987;10:127–133.